Quantitative analysis of tyramine by ion exchange chromatography

In the course of basic biochemical studies on banana plant tissue in this laboratory, it became desirable to have a method for the automatic quantitative determination of tyramine together with ammonia, lysine, histidine and arginine. The method of MOORE, SPACKMAN AND STEIN[1,2] has been widely used for the quantitative analysis of basic amino acids on Amberlite IR-120 resin (a strong-acid cation exchanger), but this method has not been satisfactory for the quantitative elution of tyramine. KIRSHNER AND GOODALL[3] have reported the use of Amberlite IRC-50 resin (a weak-acid cation exchanger) for the separation of certain phenolic amines and their subsequent quantitative determination. By modifying and combining these two procedures, a convenient 6-h method has been developed on the Beckman/Spinco amino acid analyzer for the quantitative measurement of tyramine as well as the basic compounds normally determined on this instrument. The very recent paper of PERRY AND SCHROEDER[4] deserves particular attention since they demonstrated the separation of many amines and unidentified bases in urine by chromatography on Amberlite CG-50 resin (chromatographic grade of IRC-50). Their technique is necessarily more complex and time-consuming than the one reported here which is only for five basic constituents commonly found in plants.

Resin and column preparation

One pound of Amberlite CG-50 type 2 resin was cycled once through the sodium and acid forms before use, according to the method of HIRS, MOORE AND STEIN[5]. After the final washing, fines were removed by repeated suspension in distilled water. The resin was then suspended in 3 l of pH 5.28 sodium citrate buffer 0.35 *N* in Na^+. Concentrated NaOH (50 % w/v) was added to the suspension frequently during the next few hours to maintain the pH at 5.28. A final pH correction was made after overnight stirring. The resin was then stored in the pH 5.28 buffer under refrigeration.

The resin was suspended in de-aerated (*i.e.* boiled) pH 5.28 buffer and poured into a water-jacketed chromatographic column (0.9 cm diameter) to a height of 40 cm. A separate circulating water bath maintained the column temperature at 63° ± 2°. Since the column was run at a relatively high temperature, it was also necessary to boil the eluting buffer (pH 5.28) before use to prevent the formation of air bubbles in the resin.

Experimental

Temperatures ranging from 23° to 85° and buffers between pH 3 and 6 were explored using a flow rate of 30 ml/h, which is standard for our instrument. Optimum resolution and separation of ammonia, lysine, histidine, arginine and tyramine was achieved with pH 5.28 buffer and a column temperature of 63°. These conditions resulted in a column pressure of 12 p.s.i.g.

Standard calibration mixtures were made up in pH 5.28 sodium citrate buffer. A four-point standard curve for tyramine can be obtained in one 9-h run on the Beckman/Spinco amino acid analyzer by stopping the elution every hour for the first three hours in order to load the column with an additional tyramine standard.

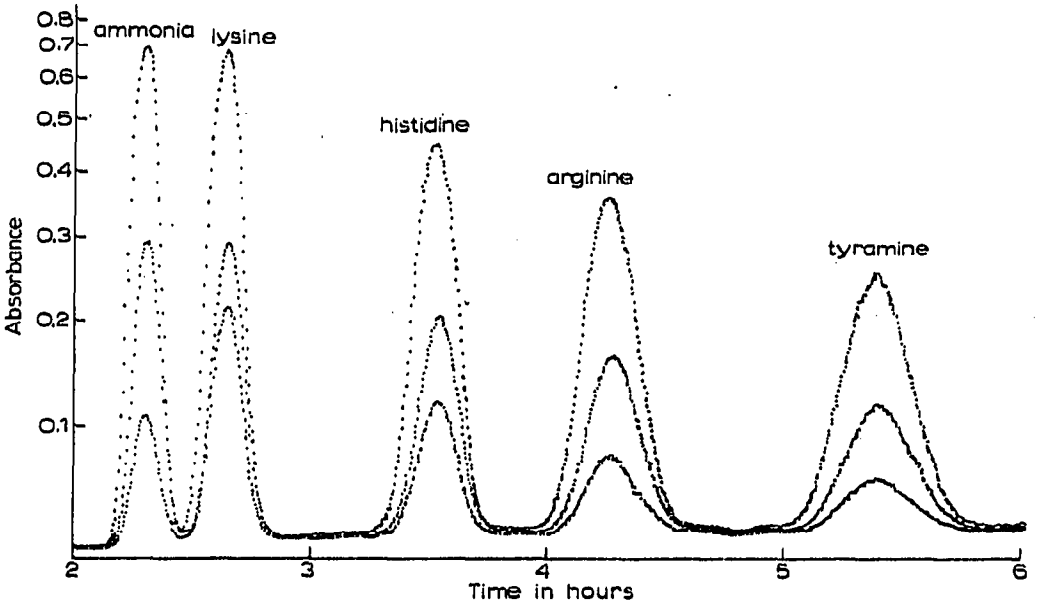

Fig. 1. Chromatographic separation of ammonia, lysine, histidine, arginine and tyramine on an Amberlite CG-50 (type 2) column and the colorimetric estimation as recorded at 570 mμ, suppressed 570 mμ and 440 mμ (lower line) by a Beckman/Spinco amino acid analyzer. Column 0.9 × 40 cm. Flow rate 30 ml/h. Temperature 63°. Pressure 12 p.s.i.g. Elution with Na citrate buffer, pH 5.28, 0.35 N in Na^+.

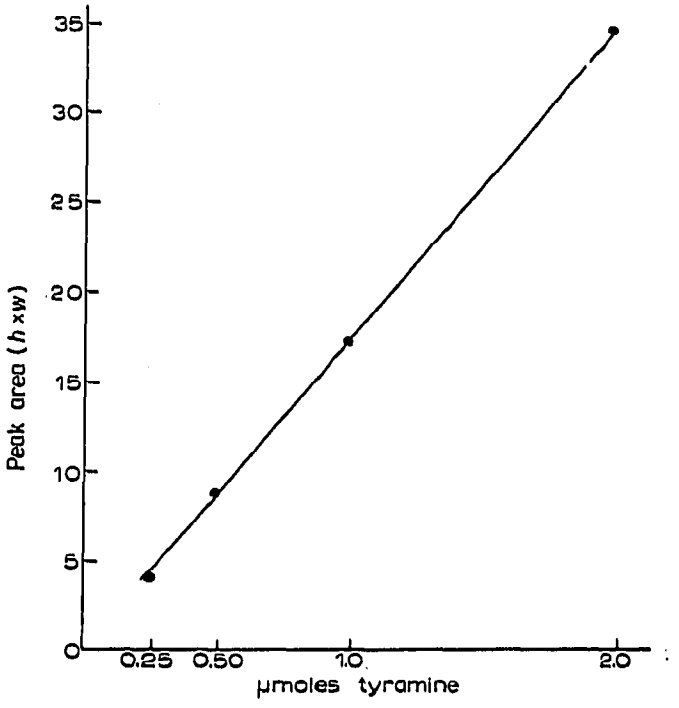

Fig. 2. Standard curve for tyramine when separated on Amberlite CG-50 (type 2) resin and recorded on a Beckman/Spinco amino acid analyzer. Tyramine constant 17.0 ± 0.3.

Results and discussion

Fig. 1 shows the chromatogram of a mixture of standard amino acids and tyramine, 1 μmole of each. Constants calculated (for 1 μmole) by the method of SPACKMAN *et al.*[2] are as follows: ammonia 19.2, lysine 22.1, histidine 18.6, arginine 18.5, tyramine 17.0. Excluding tyramine which cannot be determined on IR-120 resin, these compounds are determined as accurately (*i.e.* equal linearity and sensitivity) off CG-50 columns as off the regular IR-120 resin. The linearity of tyramine in the range 0.25 to 2.0 μmoles is shown in Fig. 2. Analyses of 10 standard samples on the same column resulted in a tyramine constant of 17.0 ± 0.3, indicating a reproducibility of better than 2 %.

Our early efforts to modify the procedure of MOORE, SPACKMAN AND STEIN[1] by changing the temperature, column length, flow rate and pH of the buffer did not improve the results for tyramine. Combining certain features of the method of KIRSHNER AND GOODALL[3] with those of the above method, however, has resulted in a relatively rapid technique for the quantitative determination of tyramine together with ammonia, lysine, histidine and arginine. Application of this procedure required only two changes in the normal operation of the Beckman/Spinco amino acid analyzer: (a) the use of CG-50 resin in place of IR-120 resin and (b) a column temperature of 63° instead of 50°. We have used this technique for the analysis of acidified (0.1 *N* HCl) 80 % ethanol extracts of banana plant tissues which were concentrated to remove ethanol, adjusted between pH 3 and 5.5, and brought to volume with water. When standard amounts of tyramine were added during the homogenization of the tissue, recoveries of tyramine were 96 ± 3 %. No regeneration of the column was necessary. However, the top few centimeters of resin were replaced after each analysis if the plant extracts contained appreciable amounts of tannins and pigments. When an analysis of tyramine alone was desired, changes in column length to 10 cm and temperature to 25° permitted a complete quantitative determination in less than 2 h.

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Relative detector response in gas chromatography

V. Halogenoalkanes, aliphatic aldehydes, pyridines

The investigation of the relative detector response of a thermal conductivity detector to organic compounds of various types, when nitrogen is the carrier gas, is continued with a study of relative responses to members of the homologous series halogenoalkanes, aliphatic aldehydes and pyridines.